16 STIMULANT METHODOLOGY

16.1 Brief Pharmacology: Central nervous system stimulants and appetite suppressants which are commonly refered to as "uppers".

16.2 Drug Group Examples: Amphetamine, methamphetamine, phentermine, phendimetrazine, phenmetrazine, methcathinone and methylphenidate.

16.3 Types of Samples:

16.3.1 Many stimulants are found in pharmaceutical preparations.

16.3.2 Methamphetamine, in particular, is often clandestinely manufactured.

16.4 Scheduling:

- Schedule I - methcathinone
- Schedule II - amphetamine, methamphetamine, phenmetrazine and methylphenidate
- Schedule III - phendimetrazine
- Schedule IV - phentermine
- Non-scheduled – ephedrine and psuedoephedrine

16.5 Extraction:

16.5.1 May be extracted from basic aqueous solutions with organic solvents. This is routinely necessary to obtain good chromatographic results with the phenethylamine-type compounds.

16.5.2 May be dry extracted with Methanol.

16.6 Color Tests Results:

16.6.1 Marquis Results

- Most phenethylamines - Orange → Brown
- Phentermine, phenmetrazine and phendimetrazine - do not give an orange color
- Add water to the well after noting color results and place under longwave UV. Methamphetamine fluoresces blue while MDMA will not.

16.6.2 Nitroprusside (Fiegel's Test) Results

- Secondary amines - dark blue

16.6.3 TBPEE Results

- Primary amines - purple
- Secondary amines - blue
- Tertiary amines - red

16.7 TLC:

16.7.1 Extraction of the sample may be necessary to get good TLC results.

16.7.2 Baths:

- TLC1, TLC2, TLC3, TLC4 and TLC5 are recommended.
- TLC9 separates methamphetamine from MDMA.
- TLC13 separates ephedrine from psuedoephedrine.

16.7.3 Detection sprays:

16.7.3.1 Fluorescamine (Fluram) for primary amines.

16.7.3.2 Iodoplatinate for secondary and tertiary amines. Iodoplatinate results may be enhanced by overspraying with ceric sulfate.

16.7.3.3 Dragendorff

16.7.3.4 Ninhydrin is recommended for ephedrine and psuedoephedrine.

16.8 UV:

16.8.1 Extraction of the sample may be necessary to get a good UV spectrum.

16.8.2 Results: Phenethylamine-type compounds give a triplet benzenoid spectrum with associated minima. (e.g., amphetamine - max. 251.5, 257, and 263 nm)

16.9 GC:

16.9.1 Extraction of the sample may be necessary to obtain good chromatography.

16.9.2 Acetyl Derivative, to improve chromatographic performance, if necessary: The acetyl derivative of phenethylamines is made by drawing up 1 µL of sample followed by 1 µL of acetic anhydride, separated by an air bubble. The acetyl derivative should have a longer retention time than the underivatized compound and may require a higher chromatographic temperature than the underivatized compound.

16.10 GC/MS:

16.10.1 The concentration of the sample must be strong enough to detect the $[M-H]^+$ ion and its associated ^{13}C isotope peak/molecular in order for the result to be considered definitive. (e.g., methamphetamine must have a 148 and 149 *m/z* ion)

16.11 FTIR:

16.11.1 Extraction from excipients may be necessary to obtain a good spectrum or chromatographic performance.

16.11.2 GC-FTIR is a useful technique to differentiate between phenethylamine-type compounds.

16.12 Amphetamine/Methamphetamine Quantitation:

16.12.1 See GC section 10 for general quantitation procedure.

16.12.2 Reagents:

- Methylene Chloride or Chloroform
- Amphetamine Sulfate (primary standard)
- Methamphetamine HCl (primary standard)
- Tridecane

- 20% NaOH solution

16.12.3 Internal Standard Solution:

16.12.3.1 Prepare a sufficient volume to dilute the standard solutions and all samples.

16.12.3.2 Prepare a 1 mg/mL solution of tridecane in methylene chloride or chloroform in the appropriate volumetric flask.

16.12.4 Standard Solution:

16.12.4.1 Prepare a 2 mg/mL standard of the appropriate material by weighing 20 mg of the desired material, diluting to 10 mL with water and making basic with 20% sodium hydroxide until pH is above 10. Extract with an equal volume of internal standard solution in a separatory funnel.

16.12.4.2 Prepare a solution of another concentration within the linear range in the same manner to use as the check standard.

16.12.5 Sample Solution:

Verify the identity of the salt originally present in the sample by suitable chemical testing. Sample weight should be adjusted accordingly. Accurately weigh approximately 20 mg (or more) of the sample, dilute to 10 mL with water and extract as with the standard solution.

16.12.6 GC Analysis:

16.12.6.1 GC parameters:

- Column: 15 m HP-1 or HP-5 capillary (0.25 mm i.d, 0.25 μm film thickness)
- Oven temperature: 70 - 210ºC at 20ºC per minute
- FID temperature : 270ºC

16.13 Differentiation of the Stereoisomers of Methamphetamine using GC Derivatization (Determination of "ICE"):

16.13.1 "Ice" is a crystalline form of nearly pure d-methamphetamine.

16.13.2 Isomer determination is not required for normal analysis, but may be requested by an agency to provide information as to the manufacturing process.

16.13.3 Procedure:

16.13.3.1 Samples of methamphetamine should be dissolved in $CHCl_3$ or CH_2Cl_2 for GC analysis. Extraction is not usually necessary.

16.13.3.2 Standards, consisting of d or l (optically pure) methamphetamine and the d, l -racemate should be prepared in $CHCl_3$ or CH_2Cl_2 at concentrations of approximately 1-2 mg/mL. It is not necessary to use both optically pure isomer standards.

16.13.3.3 n-Trifluoroacetyl-l-prolylchloride (l -TPC) may be obtained from Regis Chemical Co. (Chicago, Il.) or Sigma/Aldrich. l -TPC is supplied as 0.1M in $CHCl_3$ with 1-2% of the d isomer (d-TPC).

16.13.3.4 GC conditions:

- Injection port: 270º C
- Detector: 280º C;
- Oven: 215º C isothermal
- Split flow: approx 100:1 (standard split liner)
- Columns:
- HP-1 (Methyl silicone) 0.25 mm x 15 m x 0.25 µm (i.d. x length x film thickness)
- HP-5 (5% Phenyl methyl silicone) 0.25 mm x 15 m x 0.25 µm
- Carrier gas: helium

16.13.3.5 Both the optically pure and the racemate standards need to be injected. The racemate will check the resolution of the chromatographic system and the optically pure standard will determine the peak of interest. Baseline resolution should occur with the racemate/ l -TPC derivatives.

16.13.3.6 Load a 10 µL syringe with 1.0 µL l -TPC, 0.5 µL air and 1.0 µL methamphetamine solution (sample or std). Inject directly into the GC.

16.13.3.7 On both columns, the l - methamphetamine / l -TPC derivative elutes first.

16.13.3.8 Several additional peaks may be seen in the chromatogram. One such peak, occurring at a retention time approximately one minute prior to the l - methamphetamine / l -TPC peaks, is due to excess l -TPC. As methamphetamine concentration increases, this peak will decrease in height. Other peaks, very close to the solvent front, appear to be due to decomposition of the l -TPC reagent.

16.13.4 Methamphetamine isomers are to not to be routinely reported on the certificate of analysis.

16.13.5 References:

16.13.5.1 Fitzgerald, R.L., et. al., "Resolution of Methamphetamine Stereoisomers in Urine Drug Testing: Urinary Excretion of R(-)-Methamphetamine Following use of Nasal Inhalers", *J. Anal. Tox*., Vol 12, Sept/Oct 1988, pp. 255-259.

16.13.5.2 Fitzgerald, R.L., et. al., "Determination of 3,4-Methylenedioxyamphetamine and 3,4-Methylenedioxymethamphetamine Enantiomers in Whole Blood", *J. Chromatogr*., 490 (1989), pp. 59-69.

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